



The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item	Recommendation	Section/line number, or reason for not reporting
Study design	1 For each experiment, provide brief details of study design including: - The groups being compared, including control groups. If no control group has been used, the rationale should be stated. - The experimental unit (e.g. a single animal, litter, or cage of animals).	Line 626-696 in the Methods section Study 1: Control group: DON group (3mg of DON per kg) for 4 weeks. Study 2: Control group: AFB1 group (60 µg of aflatoxin B1 daily) for 12 days. Study 3: Control group: ABX group (chitosan-coated lactulose solution) Each animal serves as the experimental unit. Study 1: 6 animals per group Study 2: 6 animals per group Study 3: 6 animals per group Study 4: 6 animals per group
Sample size	2 - Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. - Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	The animals in this study were sourced from larger experiments conducted by our group as four separate studies. Study 1 consisted of 12 piglets; Study 2 consisted of 12 piglets; study 3 also consisted of 12 piglets; study 4 had 16 piglets. Each divided into 2 groups. A total of 62 pigs were used. A power analysis for physiological parameters such as body weight, diarrhea index, e.t.c was performed using two-sample t-tests, with a target power of at least 0.80.
Inclusion and exclusion criteria	3 - Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i>. If no criteria were set, state this explicitly. - For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. - For each analysis, report the exact value of <i>n</i> in each experimental group.	Animals were selected and included based on their initial body weight Data were excluded from analyses if they were classified as outliers (values exceeding 2 standard deviations from the respective group mean) or if samples failed . . . Figure 1, 2, 3, 5, 6, 7 and Supplementary figures show the number of animals in the 4 studies. n=4-8
Randomisation	4 - State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. - Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	After the adaptation period in each study, piglets were randomised using the random number generator function on GraphPad Prism Treatment order was not considered a confounding factor in any of the four studies, as each study was conducted independently under identical housing conditions, with the same number of cages and consistent cage placement across all treatment groups within each study.
Blinding	5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Those who conducted the animal experiments were aware of the group assignments during treatment, while all those who performed every downstream assessments were not aware of the group allocation.
Outcome measures	6 - Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). - For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	This study was a hypothesis-testing study. We used a two-sided hypothesis test. The primary outcome measures were body weight and diarrhea index in our study. Sample sizes were determined using a power analysis based on two-sample t-tests with a target power of 0.80. The primary outcome measures were body weight and diarrhea index in our study. Sample sizes were determined using a power analysis based on two-sample t-tests with a target power of 0.80.
Statistical methods	7 - Provide details of the statistical methods used for each analysis, including software used. - Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Statistical analysis was performed using GraphPad Prism 8.0.1 (GraphPad Software, San Diego, CA, USA). The Shapiro-Wilk test was used to assess the normality of continuous variables. For non-normally distributed data, the Mann-Whitney U test was used. Where applicable, P-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure. Normality of continuous variables was assessed using the Shapiro-Wilk test. Differences between the two groups for normally distributed variables were assessed using a two-tailed Student's t-test. For non-normally distributed data, the Mann-Whitney U test was used. Where applicable, P-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure.
Experimental animals	8 - Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. - Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Study 1: Weaned male piglets (Duroc x Landrace x Yorkshire) at 21 days of age were used. Study 2: Suckling male piglets (Duroc x Landrace x Yorkshire) at 21 days of age were used. Study 3: Weaned male piglets at 21 days of age were used. Study 4: Weaned male piglets (Duroc x Landrace x Yorkshire) at 28 days of age were used. Their detailed weights are provided in the Animals and sample collection section of the Methods. In Study 1, animals in the DON group were fed a basal diet artificially contaminated using Fusarium infected cultures. In Study 2, the AFB group received standard milk powder and a daily oral gavage of AFB. All pigs had ad libitum access to water, and those in Studies 1, 2 and 4 had ad libitum access to feed, while Study 2 piglets received milk replacer ad libitum
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: - What was done, how it was done and what was used. - When and how often. - Where (including detail of any acclimatisation periods). - Why (provide rationale for procedures).	Study 1: Animals were acclimated to the experimental conditions for 7 days before the start of the experiment. They were then divided into two groups: Control and Treatment. The Control group received standard milk powder and water ad libitum. The Treatment group received standard milk powder and water ad libitum, plus a daily oral gavage of DON (3 mg/kg) for 4 weeks. The animals were then sacrificed and tissues collected for analysis. Study 2: Animals were acclimated to the experimental conditions for 7 days before the start of the experiment. They were then divided into two groups: Control and Treatment. The Control group received standard milk powder and water ad libitum. The Treatment group received standard milk powder and water ad libitum, plus a daily oral gavage of AFB (60 µg/kg) for 12 days. The animals were then sacrificed and tissues collected for analysis. Study 3: Animals were acclimated to the experimental conditions for 7 days before the start of the experiment. They were then divided into two groups: Control and Treatment. The Control group received standard milk powder and water ad libitum. The Treatment group received standard milk powder and water ad libitum, plus a daily oral gavage of ABX (chitosan-coated lactulose solution) for 12 days. The animals were then sacrificed and tissues collected for analysis. Study 4: Animals were acclimated to the experimental conditions for 7 days before the start of the experiment. They were then divided into two groups: Control and Treatment. The Control group received standard milk powder and water ad libitum. The Treatment group received standard milk powder and water ad libitum, plus a daily oral gavage of AFB (60 µg/kg) for 12 days. The animals were then sacrificed and tissues collected for analysis.
Results	10 For each experiment conducted, including independent replications, report: - Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). - If applicable, the effect size with a confidence interval.	Effect sizes and confidence intervals were not reported. The analysis relied on p values from unpaired t tests for comparisons between control and treatment groups. For adjusted p values for high throughput data (microbiota, metabolomics, metabolomics). Data are presented as mean ± SEM for bar plots and as median with interquartile ranges (IQR) for box plots.

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Line 3-19 in abstract section
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Lines 2-79 in the Introduction section The pig model closely mimics human gastrointestinal physiology. DON, AFB, ABX, and AAD models together capture mycotoxin toxicity, antibiotic perturbation, and dysbiosis, providing a comprehensive platform for studying gut microbial and metabolic dysfunction relevant to human health and food security.
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	All animal procedures were performed in accordance with the guidelines of the Animal Care and Use Committee of Yangzhou University and were approved under Clinical Permit No. SYXK (SU) IACUC 2021-0026.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	1. Human endpoints were clearly defined. Pigs showing >20% body weight loss, inactivity, distress, severe lethargy, or inability to access food/water were euthanized and autopsied for histological examination. 2. Supportive care: All pigs were provided with ad libitum access to water, soft bedding, and daily enrichment (e.g., straw bedding, social interaction, and enrichment toys). 3. Monitoring: Body weight and health condition (activity, feed consumption) were monitored daily for each pig, particularly for those in the mycotoxin and antibiotic-perturbation groups. Mild to moderate diarrhoea occurred in the AFB-exposed suckling piglets and in the antibiotic-associated diarrhoea group. It was managed through supportive care and daily health monitoring. Humane endpoints were strictly enforced to minimize suffering. Euthanasia was performed using lethal agents (CO₂ asphyxiation) followed by exsanguination. Before starting experiments to generate data, reliability to assess food or water, prolonged inability to stand (not to move) during handling, >10% gross body fluid volume loss, dehydration, or signs of severe distress. Pigs were monitored daily for body weight, feed consumption, activity level, and overall health status. During peak treatment periods, interventions were conducted based on signs of welfare signs of pain or distress, including reduced appetite, abnormal posture, and vocalization.
Interpretation/scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Lines 425-593 in the Discussion section
Generalisability/translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Our findings establish a multi-kingdom framework for mycotoxin toxicity, positioning the mycobiota, particularly <i>K. sinensis</i> and <i>poA</i> , as key ecological and molecular nodes linking dysbiosis to functional impairment with implications for mitigating DON-induced losses in livestock and improving global food safety.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	A detailed experimental protocol outlining the research question and study designs for the four separate studies was prepared before the start of the experiments. The piglets used in this study were drawn from larger experiments conducted by our group. This protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Yangzhou University (SYXK (SU) 2021-0026). While the protocol was not prospectively registered in a public repository, the full methodology is described in detail within this manuscript.
Data access	20	Provide a statement describing if and where study data are available.	
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	There is no financial or non-financial competing interests. This work was supported by the National Key R&D Program of China under 2023YFD1801100, the National Key R&D Program of China under 2023YFD1801200, the Natural Science Foundation of China under 32202717 and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).